

ANTIBACTERIAL COMPOSITIONSField of the Invention

5 The present invention relates to (alkyl)aminopyrrolizidine compounds and to their use in medicine. In particular, the invention relates to the use of (alkyl)aminopyrrolizidine compounds as antibacterial drugs.

Background to the Invention

10 Many bacterial infections are presently effectively treated with antibiotics and prevented with various vaccines. However, bacterial infections still cause large numbers of fatalities. In particular, the rise in the incidence of multi-drug resistant bacterial infections has made the need for alternative means of treatment more pressing, while the use of anthrax spores as a biological weapon has focused attention on the need for widely available and effective antimicrobial agents effective against *Bacillus anthracis*.

15 The number of nosocomial infections due to antibiotic resistant bacteria has increased sharply in recent years. Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as one of the main causative agents. MRSA infections are presently treated with the glycopeptide antibiotic vancomycin. However, there are indications that the continued use of vancomycin to treat MRSA infections could give rise to a fully glycopeptide resistant population of *Staphylococcus aureus*. Thus, there is a need for further antimicrobial agents for the treatment of antibiotic resistant bacterial infections.

20 Anthrax is caused by the bacterium *Bacillus anthracis*. While anthrax commonly affects hooved animals such as sheep and goats, humans may also acquire this disease. Historically, the principal risk factor for inhalation anthrax is the inhalation of anthrax spores from industrial processes such hide tanning and wool processing. However, anthrax has recently been used as a biological weapon in bio-terrorism.

25 There are usually two stages of inhalation anthrax. The first stage can last from hours to a few days and is similar to a flu-like illness with fever, headache, cough, shortness of breath, and chest pain. The second stage often develops suddenly and is characterized by shortness of breath, fever, and shock. The prognosis of inhalation anthrax once it reaches the second stage is poor, even with antibiotic therapy and up to 90% of cases in the second stage are fatal.

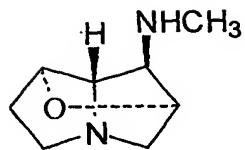
30 There is therefore a need for further antimicrobial agents effective in the treatment of anthrax.

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Summary of the Invention

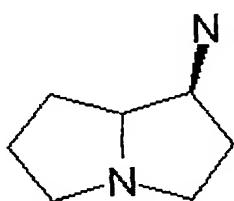
40 According to a first aspect of the invention there is provided an antibacterial (alkyl)aminopyrrolizidine compound, which compound is: (a) for use in therapy or prophylaxis, and/or (b) in a pharmaceutical composition, and/or (c) in a unit dosage form, and/or (d) in a form suitable for local or systemic administration.

The compound may be a pharmaceutically acceptable derivative of loline:



Loline is a naturally-occurring alkaloid found, for example, in certain grasses.

- 5 Preferably, the compound has a saturated or unsaturated (alkyl)aminopyrrolizidine nucleus of formula:



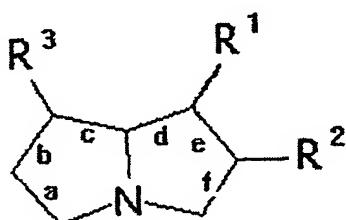
Alternatively, the compound may have a saturated or unsaturated (alkyl)aminopyrrolizidine nucleus of formula:

10



In preferred embodiments, the compounds are hydroxylated. Particularly preferred are compounds which are mono- or dihydroxylated.

- 15 In a second aspect the invention provides a compound having the formula:



- in which a, b, c, d, e and f indicate the location of optional C=C double bonds, provided, however, that the double bonds are not adjacent and that when one or more double bond(s) are present then the substitution patterns around such bonds do not violate double bond valency, wherein R¹ is amino or alkyl amino, R² and R³ are independently selected from hydrogen, oxo, halo, hydroxy and alkoxy and wherein the compound is:
- 20 (a) for use in therapy or prophylaxis; and/or
 (a) in isolated or purified form; and/or

- (b) in a pharmaceutical composition; and/or
- (c) in a unit dosage form; and/or
- (d) in a form suitable for local or systemic administration,

5 or a pharmaceutically acceptable salt or derivative thereof.

The alkyl amino is preferably C₁-C₁₀ alkyl amino (for example, C₁-C₆ alkyl amino, e.g. C₁-C₄ alkyl amino). The alkoxy is preferably C₁-C₁₀ alkoxy (for example, C₁-C₆ alkoxy, e.g. C₁-C₄ alkoxy).

10 The alkyl amino may be a C₁, C₂, C₃, C₄, C₅ or C₈ alkyl amino, while the alkoxy may be a C₁, C₂, C₃, C₄, C₅ or C₆ alkoxy. The halo may be chloro, fluoro, iodo or bromo, preferably chloro, fluoro or iodo.

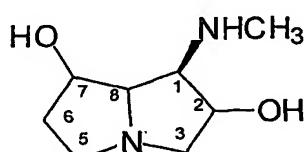
The compounds contemplated according to the second aspect of the invention may be saturated or unsaturated.

15 Preferred unsaturated compounds of the invention are 1,2-dehydro-, 5,6-dehydro-, 6,7-dehydro or 7,8-dehydro.

Preferred are compounds in which R² and/or R³ are hydroxy and/or wherein R¹ is amino or C₁ alkyl amino (methylamino).

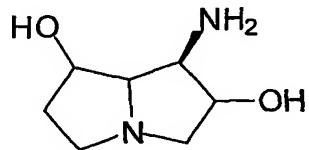
20 Particularly preferred are compounds selected from:

- (a) 2,7-dihydroxy-1-methylaminopyrrolizidine:



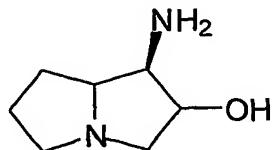
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- (b) 2,7-dihydroxy-1-aminopyrrolizidine:

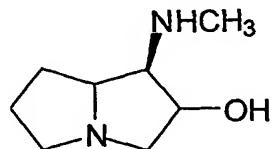


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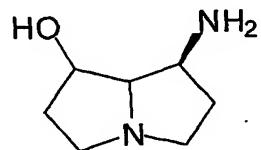
- (c) 2-hydroxy-1-aminopyrrolizidine:



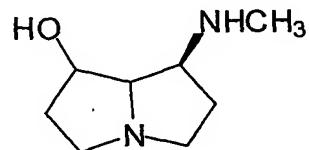
5 (d) 2-hydroxy-1-methylaminopyrrolizidine:



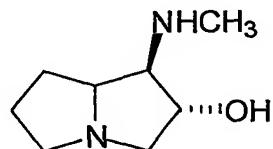
5 (e) 7-hydroxy-1-aminopyrrolizidine:



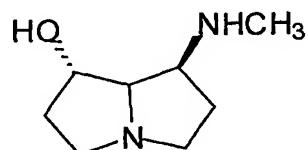
10 (f) 7-hydroxy-1-methylaminopyrrolizidine:



15 (g) 1 α -methylamino-2 β -hydroxypyrrrolizidine:



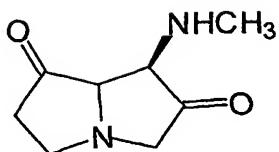
20 (h) 1 α -methylamino-7 β -hydroxypyrrrolizidine:



- 20 (i) 1 α -amino-2 β -hydroxypyrrrolizidine;
 (j) 1 α -amino-7 β -hydroxypyrrrolizidine;
 (k) 1 α -amino-2,7 β -hydroxypyrrrolizidine;
 (l) 1 α -methylamino-2,7 β -hydroxypyrrrolizidine.

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Also preferred are compounds wherein R¹ is C₁ alkyl amino (methylamino) and R² and R³ are oxo. Such compounds have the formula:



Other preferred compounds are saturated wherein R¹, R² and R³ are as shown below:

5

R ¹	R ²	R ³
Amino	Hydrogen	Hydrogen
Amino	Hydrogen	Oxo
Amino	Hydrogen	Hydroxy
Amino	Hydrogen	Halo
Amino	Hydrogen	Alkoxy
Amino	Oxo	Hydrogen
Amino	Oxo	Oxo
Amino	Oxo	Hydroxy
Amino	Oxo	Halo
Amino	Oxo	Alkoxy
Amino	Hydroxy	Hydrogen
Amino	Hydroxy	Oxo
Amino	Hydroxy	Hydroxy
Amino	Hydroxy	Halo
Amino	Hydroxy	Alkoxy
Amino	Halo	Hydrogen
Amino	Halo	Oxo
Amino	Halo	Hydroxy
Amino	Halo	Halo
Amino	Halo	Alkoxy
Amino	Alkoxy	Hydrogen
Amino	Alkoxy	Oxo
Amino	Alkoxy	Hydroxy
Amino	Alkoxy	Halo
Amino	Alkoxy	Alkoxy
Methylamino	Hydrogen	Hydrogen
Methylamino	Hydrogen	Oxo
Methylamino	Hydrogen	Hydroxy
Methylamino	Hydrogen	Halo
Methylamino	Hydrogen	Alkoxy
Methylamino	Oxo	Hydrogen
Methylamino	Oxo	Oxo
Methylamino	Oxo	Hydroxy

Methylamino	Oxo	Halo
Methylamino	Oxo	Alkoxy
Methylamino	Hydroxy	Hydrogen
Methylamino	Hydroxy	Oxo
Methylamino	Hydroxy	Hydroxy
Methylamino	Hydroxy	Halo
Methylamino	Hydroxy	Alkoxy
Methylamino	Halo	Hydrogen
Methylamino	Halo	Oxo
Methylamino	Halo	Hydroxy
Methylamino	Halo	Halo
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Alkylamino	Alkoxy	Hydrogen
Alkylamino	Alkoxy	Oxo
Alkylamino	Alkoxy	Hydroxy
Alkylamino	Alkoxy	Halo
Alkylamino	Alkoxy	Alkoxy

Unsaturated compounds according to the invention may also have R¹, R² and R³ as shown in the table above. Such compounds may, for example, be 1,2-dehydro-, 5,6-dehydro-, 6,7-dehydro or 7,8-dehydro.

- 5 In the compounds listed in the table above, the alkoxy is preferably C₁, C₂, C₃, C₄, C₅ or C₆ alkoxy, the alkylamino is preferably C₁, C₂, C₃, C₄, C₅ or C₆ alkylamino and the halo is preferably chloro, fluoro, iodo or bromo.
- 10 In another aspect, the invention provides a method of treating a bacterial infection comprising administering the compound of the invention to a patient.

Any bacterial infection may be treated using the compounds of the invention, but preferred are bacterial infections with a Gram-positive bacteria (especially low G+C Gram-positive bacteria, including *Staphylococcus* spp. or *Bacillus* spp.)

The invention finds particular utility in the treatment of infection with *S. aureus* or *S. epidermidis*.

20 The compounds of the invention may also be used to treat infection with MRSA, for example selected from any of C-MSRA1, C-MRSA2, C-MRSA3, C-MRSA4, Belgian MRSA, Swiss MRSA and any of the EMRSA strains. Accordingly, the invention therefore finds utility in the treatment or prophylaxis of infections mediated by drug-resistant bacteria and in the treatment or prophylaxis of nosocomial infections.

25 In yet other applications, the compounds of the invention are used to treat infection with *Bacillus anthracis*, and the invention therefore finds utility in the treatment or prophylaxis of anthrax.

In another aspect, the invention contemplates the use of the compounds of the invention for the manufacture of a medicament for use as an antibacterial agent.

30 In yet another aspect, the invention provides a process for the manufacture of an antibacterial agent characterized in the use of the compound of the invention.

35 In another aspect, the invention contemplates a composition comprising the compound of the invention in combination with:

- (a) an antimicrobial (e.g. antibacterial) agent; and/or
- (b) an antiviral agent; and/or
- (c) an anti-inflammatory; and/or
- 40 (d) an analgesic; and/or
- (e) an immunostimulant.

Such compositions find particular utility in adjunctive therapies.

The invention also contemplates a pharmaceutical kits of parts comprising the compound of the invention, optionally in combination with an adjunctive therapeutic agent (for example those listed as (a) to (e), above). Such kits may further comprise instructions for use in antibacterial treatment or prophylaxis.

5 In another embodiment, the invention covers wound dressings, surgical implants, disinfectant scrubs, wipes and other surgical equipment or lotions comprising the compound of the invention. The compound of the invention may also find application in methods for cleaning and/or sterilizing medical instruments, in pre-operative surgical scrubs and in operating theatre hygiene maintenance procedures.

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Detailed Description of the Invention

Definitions

15 Where used herein and unless specifically indicated otherwise, the following terms are intended to have the following meanings in addition to any broader (or narrower) meanings the terms might enjoy in the art:

20 A *pharmaceutical composition* is a solid or liquid composition in a form, concentration and level of purity suitable for administration to a patient (e.g. a human or animal patient) upon which administration it can elicit the desired physiological changes. Preferably, the pharmaceutical compositions of the invention comprise the compound of the invention together with a pharmaceutical excipient.

25 The term *isolated* as applied to the compounds of the invention is used herein to indicate that the compound exists in a physical milieu distinct from that in which it occurs in nature. For example, the isolated material may be substantially isolated (for example purified) with respect to the complex cellular milieu in which it naturally occurs. When the isolated material is purified, the absolute level of purity is not critical and those skilled in the art can readily determine appropriate levels of purity according to the use to which the material is to be put. Preferred, however, are purity levels of 90% w/w, 99% w/w or higher. In some circumstances, the isolated 30 compound forms part of a composition (for example a more or less crude extract containing many other substances) or buffer system, which may for example contain other components. In other circumstances, the isolated compound may be purified to essential homogeneity, for example as determined spectrophotometrically, by NMR or by chromatography (for example GC-MS).

35 The term *pharmaceutically acceptable derivative* as applied to the compounds of the invention define compounds which are obtained (or obtainable) by chemical derivatization of the parent compounds of the invention. The pharmaceutically acceptable derivatives are therefore suitable for administration to or use in contact with the tissues of humans without undue toxicity, irritation or allergic response (i.e. commensurate with a reasonable benefit/risk ratio).

40 Preferred derivatives are those obtained (or obtainable) by alkylation, esterification or acylation of the parent pyrrolizidine compounds of the invention. The derivatives may be antibacterial *per se*, or may be inactive until processed *in vivo*. In the latter case, the derivatives of the invention act as prodrugs.

Pharmaceutically acceptable derivatives include all prodrugs (see below). Particularly preferred prodrugs are ester derivatives which are esterified at one or more of the free hydroxyls and which are activated by hydrolysis *in vivo*. The pharmaceutically acceptable derivatives of the invention retain some or all of the antibacterial activity of the parent compound. In some cases, the antibacterial activity is increased by derivatization.

5 Derivatization may also augment other biological activities of the compound, for example bioavailability. The term is to be understood *mutatis mutandis* when applied to loline.

As used herein, the term *prodrug* is intended to define any covalently bonded carriers which release the 10 antibacterial compounds of the invention *in vivo* when the prodrug is administered. Prodrugs may be prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either in routine manipulation *ex vivo* prior to administration or *in vivo*, to the antibacterial compound of the 15 invention. Prodrugs therefore include compounds of the invention wherein a hydroxy or amino group is bonded to any group that, when the prodrug is administered, cleaves to form a free hydroxyl or free amino group, respectively. Thus, examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of alcohol and amine functional groups in the compounds of the invention.

The term *pharmaceutically acceptable salt* as applied to the compounds of the invention defines any non-toxic 20 organic or inorganic acid addition salt of the free base compounds which are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and which are commensurate with a reasonable benefit/risk ratio. Suitable pharmaceutically acceptable salts are well known 25 in the art. Examples are the salts with inorganic acids (for example hydrochloric, hydrobromic, sulphuric and phosphoric acids), organic carboxylic acids (for example acetic, propionic, glycolic, lactic, pyruvic, malonic, succinic, fumaric, malic, tartaric, citric, ascorbic, maleic, hydroxymaleic, dihydroxymaleic, benzoic, phenylacetic, 4-aminobenzoic, 4-hydroxybenzoic, anthranilic, cinnamic, salicylic, 2-phenoxybenzoic, 2-acetoxybenzoic and mandelic acid) and organic sulfonic acids (for example methanesulfonic acid and p-toluenesulfonic acid). The drugs of the invention may also be converted into salts by reaction with an alkali metal halide, for example sodium chloride, sodium iodide or lithium iodide. Preferably, the compounds of the 30 invention are converted into their salts by reaction with a stoichiometric amount of sodium chloride in the presence of a solvent such as acetone.

These salts and the free base compounds can exist in either a hydrated or a substantially anhydrous form. Crystalline forms of the compounds of the invention are also contemplated and in general the acid addition 35 salts of the compounds of the invention are crystalline materials which are soluble in water and various hydrophilic organic solvents and which in comparison to their free base forms, demonstrate higher melting points and an increased solubility.

In its broadest aspect, the present invention contemplates all optical isomers, racemic forms and diastereomers of the compounds of the invention. Those skilled in the art will appreciate that, owing to the 40 asymmetrically substituted carbon atoms that may be present in the compounds of the invention, the compounds of the invention may exist and be synthesised and/or isolated in optically active and racemic forms. Thus, references to the compounds of the present invention encompass the compounds as a mixture of diastereomers, as individual diastereomers, as a mixture of enantiomers as well as in the form of individual enantiomers.

Therefore, the present invention contemplates all optical isomers and racemic forms thereof of the compounds of the invention, and unless indicated otherwise (e.g. by use of dash-wedge structural formulae) the compounds shown herein are intended to encompass all possible optical isomers of the compounds so depicted. In cases where the stereochemical form of the compound is important for pharmaceutical utility, the

5 invention contemplates use of an isolated enantiomer.

The term *antibacterial* is used hereinto define a compound that is capable of destroying bacteria or inhibiting or preventing bacterial growth or metabolism. Those skilled in the art will be aware of many different tests for detecting and/or quantifying antibacterial activity, including for example cup plate or paper disc bioassays

10 based on the detection/measurement of zones of inhibition in seeded agar plates.

The term *adjunctive* (as applied to the use of the compounds of the invention in therapy) defines uses in which the compound is administered together with one or more other drugs, interventions, regimens or treatments (such as surgery and/or irradiation). Such adjunctive therapies may comprise the concurrent, separate or

15 sequential administration/application of the compound of the invention and the other treatment(s). Thus, in some embodiments, adjunctive use of the compound of the invention is reflected in the formulation of the pharmaceutical compositions of the invention. For example, adjunctive use may be reflected in a specific unit dosage, or in formulations in which the compound of the invention is present in admixture with the other drug(s) with which it is to be used adjunctively (or else physically associated with the other drug(s) within a single unit dose). In other embodiments, adjunctive use of the compound of the invention may be reflected in the composition of the pharmaceutical kits of the invention, wherein the compound of the invention is co-packaged (e.g. as part of an array of unit doses) with the other drug(s) with which it is to be used adjunctively. In yet other embodiments, adjunctive use of the compound of the invention may be reflected in the content of the information and/or instructions co-packaged with the compound relating to formulation and/or posology.

25

The term *surgical material* is used herein to define all materials (including equipment, compositions, tools, clothing, dressings, containers, disposables, reagents, accessories, implants and prosthetics) used in surgical operations.

30 The term *Gram-positive bacterium* is a term of art defining a particular class of bacteria that are grouped together on the basis of certain cell wall staining characteristics.

The term *low G+C Gram-positive bacterium* is a term of art defining a particular subclass class of evolutionarily related bacteria within the Gram-positives on the basis of the composition of the bases in the DNA. The

35 subclass includes *Streptococcus* spp., *Staphylococcus* spp., *Listeria* spp., *Bacillus* spp., *Clostridium* spp. and *Lactobacillus* spp.).

Medical applications

40 The invention finds application in medicine, for example in methods of therapy and prophylaxis.

The medical applications may be applied to any warm-blooded animal, including humans. The applications include veterinary applications, wherein the compounds of the invention are administered to non-human

animals, including primates, dogs, cats, horses, cattle and sheep.

The compounds of the invention are antibacterial agents. Thus, the compounds of the invention find general application as antibacterial agents. They may therefore be used in the treatment or prophylaxis or diagnosis of
5 any bacterial infection.

Posology

10 The compounds of the present invention can be administered by oral or parenteral routes, including intravenous, intramuscular, intraperitoneal, subcutaneous, transdermal, airway (aerosol), rectal, vaginal and topical (including buccal and sublingual) administration.

15 The amount of the compound administered can vary widely according to the particular dosage unit employed, the period of treatment, the age and sex of the patient treated, the nature and extent of the disorder treated, and the particular compound selected.

20 Moreover, the compounds of the invention can be used in conjunction with other agents known to be useful in the treatment of bacterial diseases, disorders or infections and in such embodiments the dose may be adjusted accordingly.

25 In general, the effective amount of the compound administered will generally range from about 0.01 mg/kg to 500 mg/kg daily. A unit dosage may contain from 0.05 to 500 mg of the compound, and can be taken one or more times per day. The compound can be administered with a pharmaceutical carrier using conventional dosage unit forms either orally, parenterally, or topically, as described below.

30 The preferred route of administration is oral administration. In general a suitable dose will be in the range of 0.01 to 500 mg per kilogram body weight of the recipient per day, preferably in the range of 0.1 to 50 mg per kilogram body weight per day and most preferably in the range 1 to 5 mg per kilogram body weight per day.

35 The desired dose is preferably presented as two, three, four, five or six or more sub-doses administered at appropriate intervals throughout the day. These sub-doses may be administered in unit dosage forms, for example, containing 0.001 to 100 mg, preferably 0.01 to 10 mg, and most preferably 0.5 to 1.0 mg of active ingredient per unit dosage form.

Formulation

40 The compositions of the invention comprise the compound of the invention, optionally together with a pharmaceutically acceptable excipient.

The compound of the invention may take any form. It may be synthetic, purified or isolated from natural sources.

When purified from a natural source, the compound of the invention may be isolated or merely purified.

In embodiments where the compound of the invention is formulated together with a pharmaceutically acceptable excipient, any suitable excipient may be used, including for example inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavouring agents, colouring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc.

The pharmaceutical compositions may take any suitable form, and include for example tablets, elixirs, capsules, solutions, suspensions, powders, granules and aerosols.

15 The pharmaceutical composition may take the form of a kit of parts, which kit may comprise the composition of the invention together with instructions for use and/or a plurality of different components in unit dosage form.

Tablets for oral use may include the compound of the invention mixed with pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, 20 flavouring agents, colouring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glycetyl monostearate or glycetyl distearate, to delay absorption in the gastrointestinal tract.

25 Capsules for oral use include hard gelatin capsules in which the compound of the invention is mixed with a solid diluent, and soft gelatin capsules wherein the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin or olive oil.

30 Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in 35 the art to be appropriate.

For intramuscular, intraperitoneal, subcutaneous and intravenous use, the compounds of the invention will generally be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity.

40 Suitable aqueous vehicles include Ringer's solution and isotonic sodium chloride. Aqueous suspensions according to the invention may include suspending agents such as cellulose derivatives, sodium alginate, polyvinylpyrrolidone and gum tragacanth, and a wetting agent such as lecithin. Suitable preservatives for

aqueous suspensions include ethyl and n-propyl p-hydroxybenzoate.

The compounds of the invention may also be presented as liposome formulations.

- 5 For oral administration the compound of the invention can be formulated into solid or liquid preparations such as capsules, pills, tablets, troches, lozenges, melts, powders, granules, solutions, suspensions, dispersions or emulsions (which solutions, suspensions dispersions or emulsions may be aqueous or non-aqueous). The solid unit dosage forms can be a capsule which can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers such as lactose, sucrose, calcium phosphate,
- 10 and cornstarch.

In another embodiment, the compounds of the invention are tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders such as acacia, cornstarch, or gelatin, disintegrating agents intended to assist the break-up and dissolution of the tablet following administration such

15 as potato starch, alginic acid, corn starch, and guar gum, lubricants intended to improve the flow of tablet granulations and to prevent the adhesion of tablet material to the surfaces of the tablet dies and punches, for example, talc, stearic acid, or magnesium, calcium, or zinc stearate, dyes, coloring agents, and flavoring agents intended to enhance the aesthetic qualities of the tablets and make them more acceptable to the patient.

- 20 Suitable excipients for use in oral liquid dosage forms include diluents such as water and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptably surfactant, suspending agent or emulsifying agent.

- 25 The compounds of the invention may also be administered parenterally, that is, subcutaneously, intravenously, intramuscularly, or interperitoneally.

In such embodiments, the compound is provided as injectable doses in a physiologically acceptable diluent together with a pharmaceutical carrier (which can be a sterile liquid or mixture of liquids). Suitable liquids

30 include water, saline, aqueous dextrose and related sugar solutions, an alcohol (such as ethanol, isopropanol, or hexadecyl alcohol), glycols (such as propylene glycol or polyethylene glycol), glycerol ketals (such as 2,2-dimethyl-1,3-dioxolane-4-methanol), ethers (such as poly(ethylene-glycol) 400), an oil, a fatty acid, a fatty acid ester or glyceride, or an acetylated fatty acid glyceride with or without the addition of a pharmaceutically acceptable surfactant (such as a soap or a detergent), suspending agent (such as pectin, carhomers,

35 methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose), or emulsifying agent and other pharmaceutically adjuvants. Suitable oils which can be used in the parenteral formulations of this invention are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, sesame oil, cottonseed oil, corn oil, olive oil, petrolatum, and mineral oil.

- 40 Suitable fatty acids include oleic acid, stearic acid, and isostearic acid. Suitable fatty acid esters are, for example, ethyl oleate and isopropyl myristate.

Suitable soaps include fatty alkali metal, ammonium, and triethanolamine salts and suitable detergents include cationic detergents, for example, dimethyl dialkyl ammonium halides, alkyl pyridinium halides, and alkylamines acetates; anionic detergents, for example, alkyl, aryl, and olefin sulphonates, alkyl, olefin, ether, and monoglyceride sulphates, and sulphosuccinates; nonionic detergents, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers; and amphoteric detergents, for example, alkyl-beta-aminopropionates, and 2-alkylimidazoline quarternary ammonium salts, as well as mixtures.

The parenteral compositions of this invention will typically contain from about 0.5 to about 25% by weight of the compound of the invention in solution. Preservatives and buffers may also be used. In order to minimize or

eliminate irritation at the site of injection, such compositions may contain a non-ionic surfactant having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations ranges from about 5 to about 15% by weight. The surfactant can be a single component having the above HLB or can be a mixture of two or more components having the desired HLB. Illustrative of surfactants used in parenteral formulations are the class of polyethylene sorbitan fatty acid esters, for example, sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol.

The compounds of the invention may also be administered topically, and when done so the carrier may suitably comprise a solution, ointment or gel base. The base, for example, may comprise one or more of the following:

petrolatum, lanolin, polyethylene glycols, bee wax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Topical formulations may contain a concentration of the pyrrolizidine compound or its pharmaceutical salt from about 0.1 to about 10% w/v (weight per unit volume).

When used adjunctively, the compounds of the invention may be formulated for use with one or more other drug(s). In particular, the compounds of the invention may be used in combination with an antimicrobial (e.g. antibacterial) agent, antiviral agent, anti-inflammatory, analgesic (e.g. opioids or NSAIDs) or immunostimulant.

Thus, adjunctive use may be reflected in a specific unit dosage designed to be compatible (or to synergize) with the other drug(s), or in formulations in which the compound is admixed with one or more of the

antimicrobial (e.g. antibacterial) agents, antiviral agents, anti-inflammatories, analgesics or immunostimulants (or else physically associated with the other drug(s) within a single unit dose). Adjunctive uses may also be reflected in the composition of the pharmaceutical kits of the invention, in which the compound of the invention is co-packaged (e.g. as part of an array of unit doses) with the antimicrobial (e.g. antibacterial) agent, antiviral agent, anti-inflammatory, analgesic or immunostimulant. Adjunctive use may also be reflected in information and/or instructions relating to the co-administration of the compound with antimicrobial agents and/or antiinflammatories.

Exemplification

The invention will now be described with reference to specific Examples. These are merely exemplary and for illustrative purposes only: they are not intended to be limiting in any way to the scope of the monopoly claimed or to the invention described.

Example 1

A semi-synthetic reaction mixture derived from loline was tested for activity by incubation for 12-24 hours at 37°C at various concentrations with a suspension of 1×10^3 c.f.u. of *Bacillus anthracis* SN1. After incubation,

- 5 test samples were plated onto solid agar plates and colonies counted after incubation at 37°C for 24 hrs.
Complete bacterial killing was observed.

Example 2

- 10 A semi-synthetic reaction mixture derived from loline was tested for activity by incubation for 12-24 hours at 37°C at various concentrations with a suspension of 1×10^3 c.f.u. of *Staphylococcus aureus*. After incubation, test samples were plated onto solid agar plates and colonies counted after incubation at 37°C for 24 hrs.
Complete bacterial killing was observed.

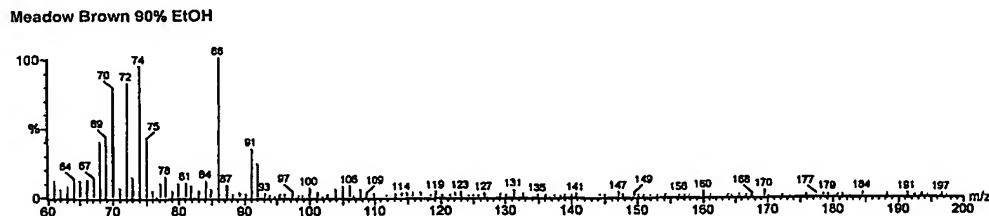
15 Example 3: Isolation of antibacterial loline derivatives

Meadow brown butterflies have activity against *Staphylococcus aureus* (MRSA) and a 50% ethanol extract of these butterflies contains the activity. Furthermore, the activity is retained by a strongly acidic cation exchange resin. The material not bound to the resin is inactive but the material displaced by 2M ammonia solution has 20 activity. This ammonia fraction contains various open-furan ring lolines (as determined by mass spectroscopy).

Two strains of MRSA were used – XU212 – an MRSA with an MDR transporter (Tet(K)) and SA1199B an MDR strain with the NorA transporter and a gyrase mutation. Meadow brown unbound (MBC) was inactive against both strains at 256 µg/ml but Meadow Brown cation exchange bound (MBbd) was inactive against XU212 at 25 256 µg/ml but active against SA1199B at 128-256 µg/ml. The observed level of activity is suppressed in these assays by the presence of other components (which dilute the active component).

The mass spectrum of a novel pyrrolizidine alkaloid in the active fraction of the Meadow Brown is shown below:

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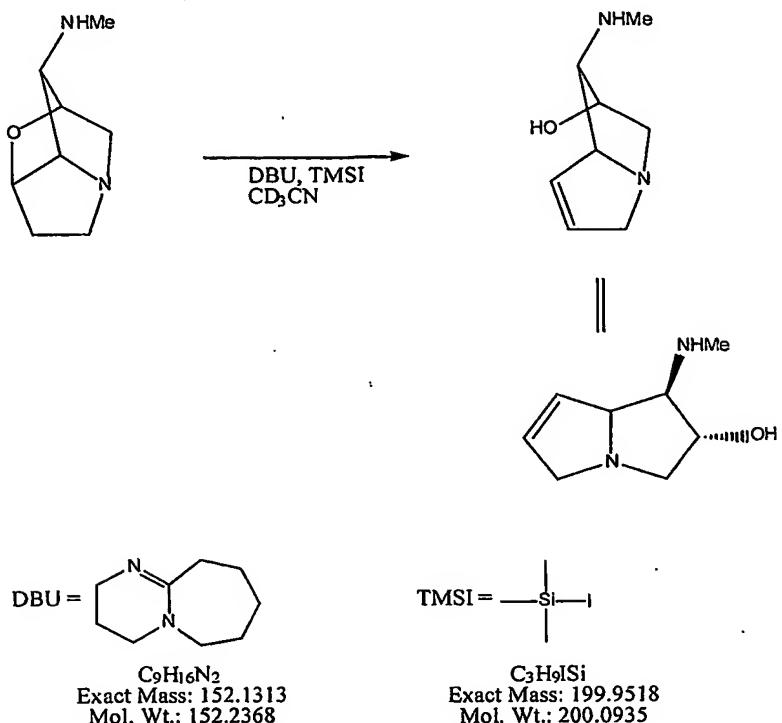


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Example 4: Synthesis of antibacterial loline derivatives

Loline hydrochloride (15 mg) was treated with DBU (2 equivalents) and TMSI (1.2 equivalents) at room temperature for 1 hour. The reaction was quenched through the addition of MeOH and the solvents were removed under reduced pressure. The crude residue was dissolved in chloroform and washed with water.

Both layers were concentrated under reduced pressure aqueous and chloroform extracts of the product as shown below:



Equivalents

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The foregoing description detail presently preferred embodiments of the present invention. Numerous modifications and variations in practice thereof are expected to occur to those skilled in the art upon consideration of these descriptions. Those modifications and variations are intended to be encompassed within the claims appended hereto.